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| <p>The objective of this research was to find how exposure to extremely low frequency (elf) electric and magnetic fields (EMFs) affects human cells. It was previously postulated that EMFs initiate a transduction cascade at the cell membrane. If this is the case, then within minutes, a sequence of events, including early gene activation, could set up the initial steps leading to proliferation. A series of experiments were set up to determine the validity of this model by measuring specific proteins or protein groups. The results showed that some proteins are increased following exposure to a 60 Hz EMF for periods from 45 to 120 minutes. Other proteins, however, are either decreased or unaffected by the presence of the field.</p> |  |   |                            |
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FINAL REPORT

Grant #: N00014-90-J-1266

PRINCIPAL INVESTIGATOR: Ann S. Henderson

INSTITUTION: Hunter College-CUNY

GRANT TITLE: Exposure of Human Cells to Electromagnetic Fields

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OBJECTIVES: To find how exposure to extremely low frequency (elf) electric and magnetic fields (EMFs) effects human cells. It is postulated that EMFs initiate a signal transduction cascade at the cell membrane leading to changes in the influx of calcium and/or receptor binding. If this is the case, then within minutes, a sequence of events, including early gene activation, could set up the initial steps leading to proliferation. A series of experiments has been set up to determine the validity of this model. The aims are to make specific comparisons between unexposed and exposed cells which include measuring protein synthetic patterns. The hypothesis that common pathways are used by cells exposed to EMFs or TPA is also tested in this study. Inherent in our approach is to develop unequivocal methods for measurement of the effects of EMFs.

APPROACH: HeLa or HL-60 cells are exposed to 60 Hz sinusoidal EMFs at 8  $\mu$ T for 20 minutes. Different experimental approaches have been used to investigate the effect of EMF exposure on polypeptide synthetic activity, enzymatic activity or protein modification. The experimental designs include setting conditions by measurement of products of transfected genes, immunoprecipitation to measure protein phosphorylation, inhibition of enzymatic activity and determinations using 2-D gel electrophoresis.

ACCOMPLISHMENTS. (1) **Phosphorylation of Fos protein.** The Fos protein becomes phosphorylated at serine residues present at the carboxyl terminus of the protein. Other studies have shown that the phosphorylation of the protein, causes the protein [in conjugation with Jun] to down-regulate its own promoter. EMF (or TPA) exposure results in an increase in the amount of modified (phosphorylated) Fos protein, with a maximum increase of 50 % following 20 minutes exposure. (2) **Expression of CAT protein in transfected HeLa cells.** Another approach is to measure CAT (linked to an appropriate upstream regulatory region of the

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c-fos or c-myc gene) protein following transfections of HeLa cells. CAT protein levels are then compared between unexposed and exposed cells; TPA exposure is used alone, as well in combination with EMF. CAT protein is maximally expressed at one hour following exposure, and this is also the time at which the maximum difference between CAT protein in unexposed and exposed cells is achieved. Irrespective of the order of exposure of cells to TPA or EMF in dual exposures, the effects were not synergistic. **(3) The role of PKC.** The most compelling evidence for the validity of a given observation is that the effect can be erased by inhibition. PKC was inhibited by chelerythreine (1  $\mu$ M). In the presence of the inhibitor, there was no increase in CAT protein in transfected HeLa cells exposed to EMFs. The ratio of membrane to cytoplasmic PKC $\alpha$  levels was compared in HL-60 cells with no exposure or exposed to TPA or EMFs. There is an increase observed at 10 minutes following either TPA treatment or EMF exposure. Although the effect of EMF exposure is much smaller than using TPA, the kinetics are the same. **(4) Optimizing conditions for studying proteins in cells exposed to EMFs.** Analysis of 2-D gels is by identification of known polypeptides and "grid analysis" of total gels using computer assistance. The results showed increases or decreases in some polypeptides from exposed cells. There was, however, variability in the results (based on control studies) which prompted us to analyze our system more carefully. Our previous protocol used a time period of 25 minutes following exposures. This turned out to be inadequate, based on studies of specific genes. We then did a very precise study over time as to when differences in specific proteins (Fos and Myc) were maximized following exposure to EMFs. The results showed that a period of one hour (following exposure) was required.

**CONCLUSIONS:** These studies: (a) developed appropriate conditions for determination of changes in specific proteins that are quantitatively changed in the presence of EMFs and (b) suggest that EMFs can effect the regulation of c-fos by post-translational modification of the gene product.

**SIGNIFICANCE:** The studies show that EMF exposure can affect proteins required in cellular regulatory pathways.

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PUBLICATIONS AND ABSTRACTS (total period of grant):

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